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1   **Removal of microalgae from seawater using Chitosan-alum/ferric chloride dual coagulations**

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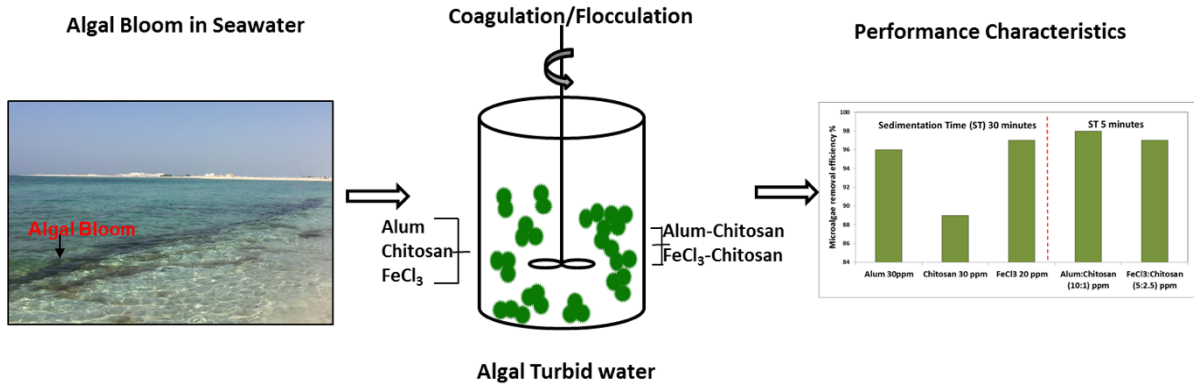4   (HBKU), Qatar Foundation, Doha, Qatar
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9   **Graphical Abstract:**
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Highlights:

- Coagulation-flocculation-sedimentation (C-F-S) experiments using $FeCl_3$ coagulant gave better process performance when compared to alum and chitosan based individual coagulations

- Dual coagulation using alum as coagulant and chitosan as flocculent aid improved microalgae removal efficiency at a reduced process time, thus making C-F-S process as attractive as Coagulation-flocculation-dissolved air flotation (C-F-D) process

- Residual alum concentration in dual coagulation process was significantly reduced when compared to alum based individual coagulation

1   **Removal of microalgae from seawater using Chitosan-alum/ferric chloride dual coagulations**

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8   **Abstract**

9   During algal bloom, it's a challenge to provide good quality feed water, and ensure sustainable RO
10   plant operations without an adequate pre-treatment of seawater. In this paper, the effectiveness of
11   the coagulation process with the individual and dual coagulants, using alum, $FeCl_3$ and chitosan,
12   were explored aiming to remove microalgae from seawater. The coagulation-flocculation-
13   sedimentation (C-F-S) experiments were conducted by optimizing multiple process strategies to
14   reduce the amounts of coagulants and also to shorten the sedimentation process time. The
15   coagulation-flocculation-dissolved air flotation C-F-D) experiments were performed to generate the
16   process data in order to evaluate the dual coagulation process performance of the C-F-S system. C-F-
17   S experiments using $FeCl_3$ coagulant gave better process performance (20 ppm $FeCl_3$ dose, 8.2 pH, 30
18   min sedimentation time and 98% microalgae removal efficiency) when compared to alum and
19   chitosan based individual coagulations. The process time of the coagulation process was significantly
20   reduced by the addition of chitosan as a flocculent aid. For dual coagulation using alum (10 ppm) as
21   coagulant and chitosan (1ppm) as flocculent aid improved microalgae removal efficiency to 98% at a
22   reduced process time of 5 minutes, making C-F-S process as attractive as C-F-D process.

25   **1 Introduction**

26   More than half of the world's population lives in water stress areas, and the numbers are
27   expected to increase to two-thirds by 2025. In many parts of the world, shortage of fresh water is a
28   looming crisis due to climate change and the increase of the global population[1]. Seawater
29   desalination is one of the feasible solutions in addressing the water crisis [2-6]. Reverse osmosis (RO)
30   is a widely used technology for desalinating seawater [7]. However, sustainable operations of sea
31   water reverse osmosis (SWRO) plants depend on the quality of the feed water. Seasonal microalgae
32   blooms are one of the operational challenges faced by SWRO operators, where blooms can hamper
33   the performance of the plant and potable water quality [8-13]. Apart from inducing particulate
34   fouling on the membrane which results in sporadic plant disruptions [14-17], microalgae has a
35   tendency to release potent toxins into the water when its cells are ruptured due to trans-membrane
36   pressure. These toxins pose severe health problems in humans [18-21], resulting in dermatologic,
37   gastrointestinal, respiratory, and neurologic disorders. The focus of this work is to improve
38   performance of pre-treatment processes to produce microalgae free feed water for SWRO plants.

39   There are several treatment options available to remove microalgae from feed water,
40   namely: (i) disinfection (ii) filtration and (iii) physico-chemical removal process. Although
41   chlorination is effective in disinfecting microalgae, the presence of residual chlorine can significantly
42   decrease the lifespan of membranes. Similar to chlorination, other disinfection methods, such as UV

and ozone, can result in cell-lysis, which releases toxins into the feed water [22]. Sand filtration/flotation processes are ineffective in removing microalgae, unless preceded by chemical coagulation-flocculation. The retention efficiency of a sand filter obtained for 145,000 cells/ml algae was reported as 80% during the first few hours of filtration and dropped to 48% after 7 hours [23]. For the coagulation process, alum and ferric chloride are among the common coagulants used by the water treatment industry. Ferric salts are preferred in sea water desalination, due to the low solubility of the resulting ferric hydroxide in seawater, over a wide range of pH. On the other hand, the use of alum as a cationic coagulant in seawater is not favoured due to the high solubility of aluminium hydroxide in seawater, which leads to the precipitative scaling of RO membranes [12]. Natural coagulants have also been used in water treatment processes [24, 25]. Chitosan, a cationic polymer prepared from crab/shrimp shells, is the second most abundant biopolymer in the world after cellulose [26]. Chitosan is positively charged, due to the protonization of amino groups in a solution which makes it attractive for a variety of binding applications.

Following coagulation-flocculation, the dissolved air flotation process (C-F-D) is commonly used in the upstream of MF/UF systems to minimize the solid loadings [27-30]. Dissolved air flotation (DAF) is a relatively quick process which is suitable for the removal of low-density algal particles. However, DAF is an energy intensive process (0.05-0.075 kWh/m^3 of treated water)[31], as the generation of air microbubbles requires compressed air requiring as high as 7 bars. Another option to remove microalgae is by the coagulation-flocculation-sedimentation (C-F-S) process. However, it is less preferred, as a long settling time is required to achieve a comparable removal rate with DAF. Sedimentation time of the C-F-S process using some common coagulants for microalgae removal was reported as more than 2 hours [32]. Optimization of the coagulation strategies can achieve the best microalgae removal rate in the shortest time possible. The sedimentation time of microalgae can be shortened by improving the size and density of the flocs. The purpose of this article is to explore the C-F-S process, and flocculation properties of the dual coagulants, alum-chitosan, and FeCl$_3$-chitosan for microalgae removal in seawater. The dual coagulation strategy was followed to minimize the coagulant dosage, in order to optimize the density of flocs, and to minimize the sedimentation time.

2 Materials and Methods:

In Qatar, the algal bloom season starts in the month of October (Figure 1B) and ends approximately around the month of April, as shown in Figure 1A. Seawater samples analysed during these months revealed that the microalgae counts were around six times higher at the start of bloom season, when compared to the April data (Table 1). Phytoplankton counts were calculated by measuring the chlorophyll intensity of each cell, using a BD Accuri C6 flow cytometer (Ann Arbor, Michigan, USA). The dual threshold triggers on FL3 (Excitation: 488 nm; Emission: 670 nm) and FL4 (Ex: 640 nm; Em: 675 ±12.5 nm) were set just above background noise. A 50 µL sample was injected at the medium fluidic settings (35 µL/min; core size 16 µm) in order to obtain the absolute cell counts. Particle size measurements were taken by using a Jorin VIPA B HiFlo analyser (Leicestershire, UK), which indicated that the microalgae size was between 2-5 µm. In addition to microalgae, macroalgal deposits were found on the shore (Figure 1 Captions 'C1 and C2'). These blooms can cause ecological, and societal impacts, including the disruption of the intake of water for cooling/desalination[12].

Table 1 Raw sea water analysis		
Date	Apr-16	Oct-16
Algal Cell Counts		
Cells per µL	18	106
pH	8.15	8.24
Turbidity NTU	0.98	3.1
Alkalinity ppm	128.8	126.8
Total Hardness		
ppm	10,150	10,209
Calcium Hardness		
ppm	1,800	1,839
TDS ppm	49,228	49,115

Figure 1: Sea water in April 2016 (clear season) and Oct 2016 (start of bloom season). Pictures C1 & C2 shows macro algae deposits during bloom season. The pictures were taken near the desalination plant in Qatar, for this project.

The seawater samples were collected from the west coast of Qatar, in bulk, to prepare the microalgae culture solution. NKP salts were added, as per the literature method [33], on a weekly basis to the sea water in order to enhance algae growth. The purpose of using a sea water culture is to reflect heavy bloom conditions, with microalgae cell counts ~$1.5x10^3$ per µl, and to maintain the consistency of the algae model solution during the testing period.

Jar tests were carried out by using a programmable apparatus (Phipps & Bird, USA) at room temperature. The tester was programmed for rapid mixing at 100 RPM for one minute; slow mixing at 30 RPM for 15 minutes; followed by sedimentation for 30 minutes [34]. Residual aluminium concentration was determined using Agilent inductively coupled plasma-mass spectrometer (ICP-MS 7500c), equipped with automatic sampler introduction and with concentric and microflow nebulization. For dual coagulation using alum, two sets of experiments were conducted as shown in Figure 2. Firstly, an alum and chitosan mixture was used as a primary coagulant, which was added during the rapid mixing and the coagulation stage. The second set of experiments were conducted using alum as the primary coagulant and chitosan as the secondary coagulant which acted as a flocculant aid. The primary coagulant was added during the rapid mixing stage, and the secondary was added during the slow mixing stage.

About 100 mL of samples were taken after the sedimentation stage for analyses. Similar experiments were conducted using $FeCl_3$ as the primary coagulant, and chitosan, as a flocculent aid.

DAF jar tests were performed using the batch jar tester Platypus DAF system, with a 2L capacity DAF saturator. For DAF particle separation experiments, coagulation and flocculation processes were followed by a 10 minute period of flotation, using a 15-30% recycling ratio at a saturation pressure of 675 kPa [28]. The operational conditions of coagulation/flocculation are similar to the sedimentation studies. The DAF-treated samples were collected via sampling ports for characterization studies (microbial counts, turbidity). The C-F-D process was performed using alum and $FeCl_3$ as coagulants, mainly to generate the process data in order to evaluate the dual coagulation process performance of the C-F-S system.
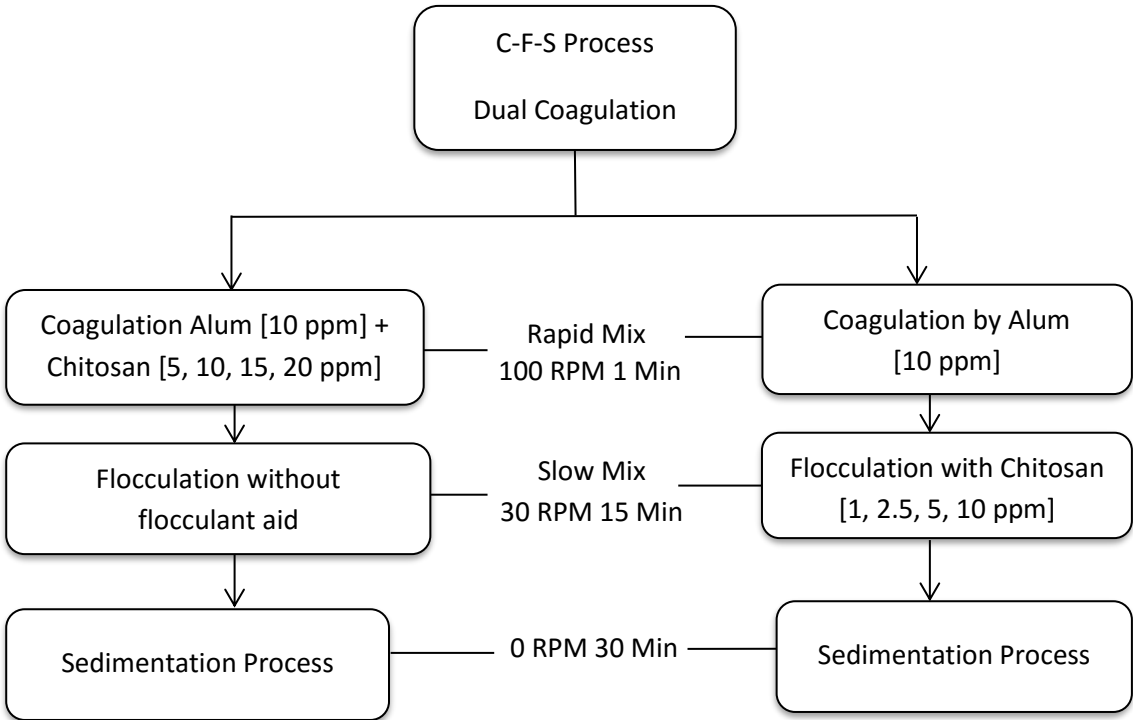

Figure 2: Flowchart indicating the stages of dual C-F-S process

3 Results and Discussion:

3.1 Coagulation studies with Alum, Chitosan and FeCl₃

The jar test experiments were conducted with alum, chitosan and ferric chloride coagulants to determine the optimal coagulant dose, based on settled water turbidity (Figure 3A). The clarified water turbidity, decreased with coagulant additions to the levels (0.7, 2.7 and 0.5 NTU for alum, chitosan and $FeCl_3$ respectively), after which turbidity increased. The optimum dose of alum, chitosan, and $FeCl_3$ for maximum turbidity removal was found to be 30, 30 and 20 mg/L respectively. The corresponding micro algae removal efficiency of alum, chitosan and $FeCl_3$ were found to be 96.3, 87.3 and 98.6 % respectively (Table 2).
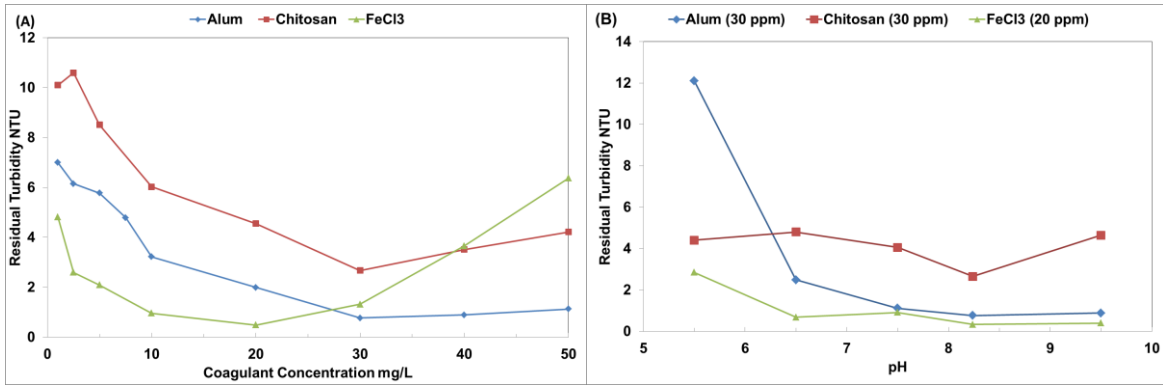
Figure: 3. Residual turbidity versus coagulant dose for alum, chitosan and ferric chloride (A); pH (B)

An overdose of alum (50 ppm) caused charge reversal which led to decrease in microalgae removal efficiency (Figure 4 A) and increase in turbidity values. An overdose of chitosan led to an increase in turbidity values, while the microalgae removal efficiency remained constant 87% (Figure 4B). Furthermore, the higher ferric chloride dose increased the turbidity values; while higher microalgae removal efficiency was observed (Figure 4E). Results show the importance of both microalgae cells counts, and turbidity measurements, to determine the extent of the coagulation process. Compared to alum and chitosan coagulants, FeCl₃ required the least amount of dosage to achieve the greatest amount of turbidity, and microalgae removal. With the optimized coagulants dose levels, the pH for maximum turbidity removal occurred at raw water with a pH of 8.2, as shown in Figure 3B. Therefore, the experiments were conducted with microalgae cultured seawater samples without pH adjustments. Clarified water was filtered using 0.45 micron filter, and characterized for residual aluminium concentration using ICP-MS. At 22°C and pH 8.2, and the residual aluminium concentration was found to be 697 μg/L. For Chitosan coagulation DOC ranges between 0.1 and 0.4 ppm.

Table2: Results of seawater (individual, dual) coagulation with coagulants alum, chitosan and FeCl₃

Coagulant Dose ppm	pH	Turbidity NTU	Microalgae Cell Counts Per μL	% Removal	Sedimentation Time Minutes	
Alum Coagulation						Residual Al ppb
Raw Water	8.2	37	1579			-
1	8.2	7	1233	21.9	30	490.1
2.5	8.2	6.15	1150	27.2	30	502.6
5	8.2	5.78	1035	34.5	30	540.8
7.5	8.2	4.79	874	44.6	30	554.2
10	8.2	3.22	270	82.9	30	590.8
20	8.2	1.99	108	93.2	30	590.7
30	8.2	0.77	58	96.3	30	697.2
40	8.2	0.89	215	86.4	30	701.3
50	8.2	1.13	818	48.2	30	750.5
30	5.5	12.1	1623	-	30	790.6
30	6.5	2.49	538	65.9	30	750.8

30	7.5	1.12	255	83.9	30	742.5
30	8.2	0.77	58	96.3	30	697.2
30	9.5	0.89	82	94.8	30	790.5
Chitosan Coagulation						**DOC ppm**
Raw Water	8.2	21.1	1827			
1	8.2	10.1	1470	19.5	30	0.1
2.5	8.2	10.6	1370	25.0	30	0.2
5	8.2	8.51	1249	31.6	30	0.2
10	8.2	6.03	588	67.8	30	0.4
20	8.2	4.55	377	79.4	30	0.2
30	8.2	2.67	274	89.6	30	0.3
40	8.2	3.51	267	89.9	30	0.2
50	8.2	4.22	273	89.6	30	0.4
20	5.5	4.41	240	90.9	30	-
20	6.5	4.8	287	89.1	30	-
20	7.5	4.06	239	90.9	30	-
20	8.2	2.66	325	87.7	30	-
20	9.5	4.65	817	69.0	30	-
Alum (A) : Chitosan (Ch; Coagulation aid)						**Residual Al ppb**
Raw Water	8.2	15	1639			
10 A:00 Ch	8.2	3.22	270	83.5	30	-
10 A:05 Ch	8.2	1.5	154	90.5	30	150
10 A:10 Ch	8.2	2.11	590	64.0	30	-
10 A:15 Ch	8.2	4	900	45.1	30	-
10 A:20 Ch	8.2	6.8	1462	10.8	30	-
Alum (A) : Chitosan (Ch; Flocculation aid)						
Raw Water	8.2	22.2	1636			-
10 A:01 Ch	8.2	1.11	39	97.6	5	67
10 A:2.5 Ch	8.2	1.5	48	97.1	5	-
10 A:05 Ch	8.2	1.21	73	95.5	5	-
10 A:10 Ch	8.2	1.22	74	95.5	5	-
FeCl₃ Coagulation						**Residual Fe ppm**
Raw Water	8.2	15.5	1430			
1	8.2	4.81	672	53.0	30	<0.1
2.5	8.2	2.6	357	75.0	30	<0.1
5	8.2	2.09	215	85.0	30	0.11
10	8.2	0.96	78	94.5	30	0.16
20	8.2	0.49	20	98.6	30	<0.1
30	8.2	1.32	16	98.9	30	0.15
40	8.2	3.66	13	99.1	30	0.82
50	8.2	6.37	8	99.4	30	2.90
20	5.5	2.85	37	97.4	30	-

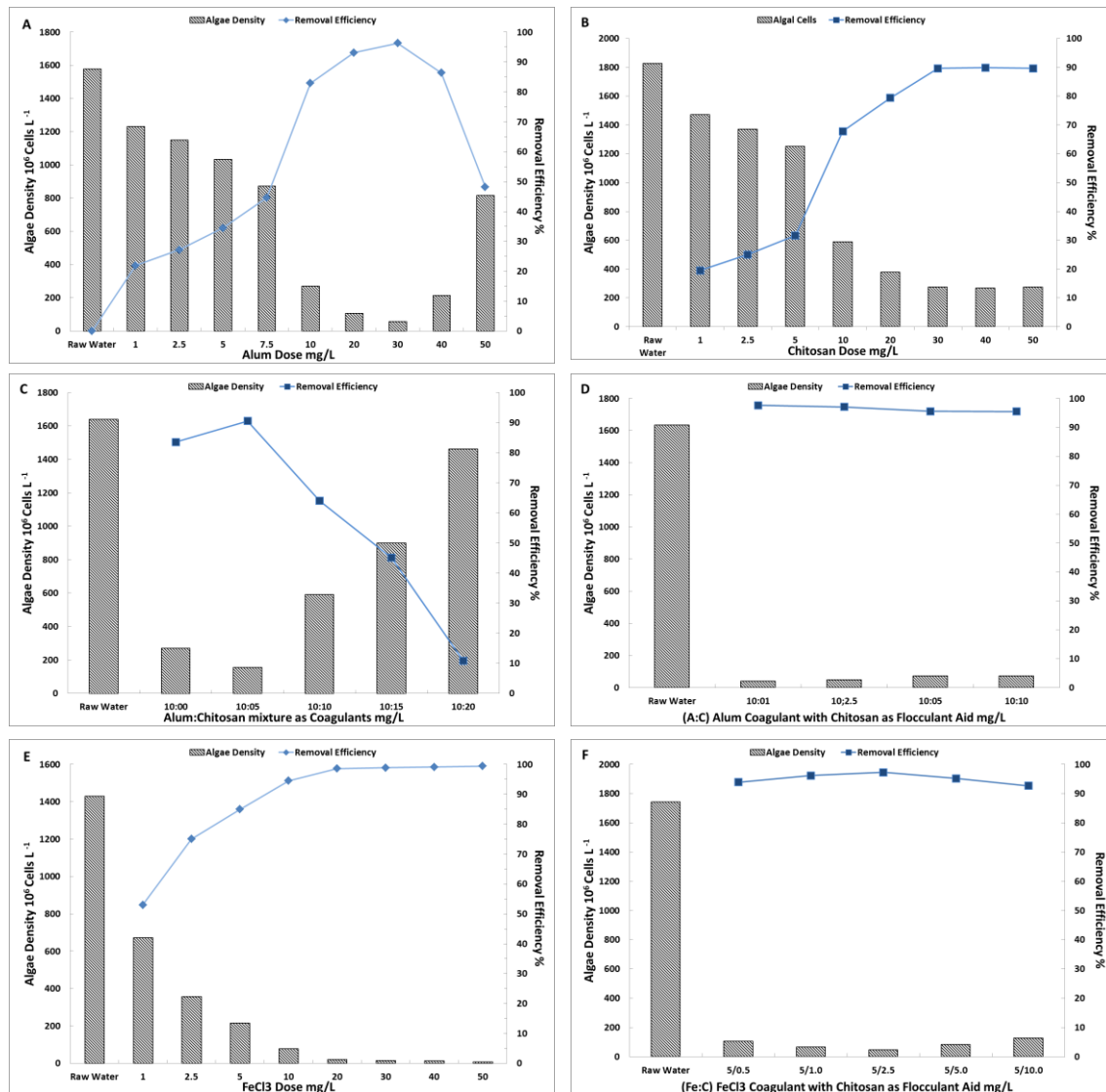| 20	6.5	0.68	34	97.6	30	-
20	7.5	0.91	52	96.4	30	<0.1
20	8.2	0.33	20	98.6	30	<0.1
20	9.5	0.4	25	98.3	30	-
FeCl$_3$ (I):Chitosan (Ch; flocculation aid)						
Raw Water	8.2	15	1744			-
5(I):0.5(Ch)	8.2	2.28	106	93.9	5	-
5(I):1.0(Ch)	8.2	2.56	67	96.1	5	-
5(I):2.5(Ch)	8.2	1.25	48	97.2	5	<0.1
5(I):5.0(Ch)	8.2	2.27	84	95.1	5	-
5(I):10(Ch)	8.2	2.05	128	92.6	5	-

1

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Figure 4: Dosage effects on coagulation performance of A: alum B. chitosan C. alum:chitosan mixture D. alum primary coagulant with chitosan as flocculent aid E. FeCl$_3$ F. FeCl$_3$ primary coagulant with chitosan as flocculent aid

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2 *3.2 Performance of C-F-D Process:*

3   In this study, the DAF performance as a particle separation method for microalgae removal was

4   investigated for alum and FeCl$_3$ coagulation processes. In the DAF process, air was added to a

5   suspension of flocculated water to induce buoyancy, which drove the floc-bubble aggregates to the

6   top of the DAF reactor towards the float layer. At the end of flotation process, clarified seawater

7   samples were collected, and characterized using flow cytometer and turbidity measurements to

8   obtain microalgae removal efficiency. It has been seen that the high ionic strength of seawater

9   affects the performance of the DAF process, and therefore the design corrections were followed to

10   maximize the DAF performance, as recommended by Edzwald [1]. The seawater saturation pressure

11   was maintained at 675 kPa, and the recycle rate was varied from 15% to 30% to optimize the DAF

12   performance, targeting microalgae removal. For alum (10 ppm) coagulation, the DAF particle

13   separation process resulted in around 94% of microalgae removal in less than two minutes of

flotation time (Table 3). Only 84% microalgae removal was achieved with 10 ppm alum concentration, using a sedimentation process with the settling time of around 30 minutes (Figure 4a). Similarly, a more efficient (ca.94%) microalgae removal was achieved for $FeCl_3$ [5 ppm] coagulation using the process, with less than two minutes flotation time as compared to the 30 minutes settling time required for C-F-S process.

Table 3 Performance of C-F-D process in microalgae removal from seawater

Coagulant	Coagulant Dose mg/L	Flotation Recycling Ratio %	Saturation Pressure kPa	Microalgae Removal Efficiency %
Alum	10	15	675	93.7
Alum	10	30	675	94.1
$FeCl_3$	5	15	675	94.7
$FeCl_3$	5	30	675	94.2

3.3 Reduction of Coagulant Dose and sedimentation time of C-F-S process through dual coagulation strategy

Dual coagulation experiments were conducted using alum and $FeCl_3$ as primary coagulants and Chitosan mainly as a coagulant/flocculant aid. Investigations were aimed to check the possibilities of reducing primary coagulant dosage and to minimize the sedimentation time. When the coagulation experiments are compared with alum at 7.5 ppm and 10 ppm dosage levels, a sharp increase (from 44% to 82%) in microalgae removal efficiency was observed (Figure 4A, Table 2). In all dual coagulation experiments involving alum, the dosage was fixed at 10 ppm. For the first set of experiments (case1) alum 10 ppm was mixed with a chitosan dosage from 1 to 10 mg/L (Figure 4C) and the mixture was added during the rapid mixing stage of the coagulation process. A maximum microalgae removal of 90.5% was achieved for alum 10 ppm: chitosan 5 ppm mixture. A further increase in chitosan concentration resulted in a sharp decrease in microalgae removal efficiency. A sedimentation time of 30 minutes was required for case 1 experiments. The re-stabilisation behaviour was characterized by the appearance of smaller flocs as shown in Figure 5C. Clarified water was filtered using 0.45 micron filter, and the residual aluminium concentration was found to be 150 μg/L.
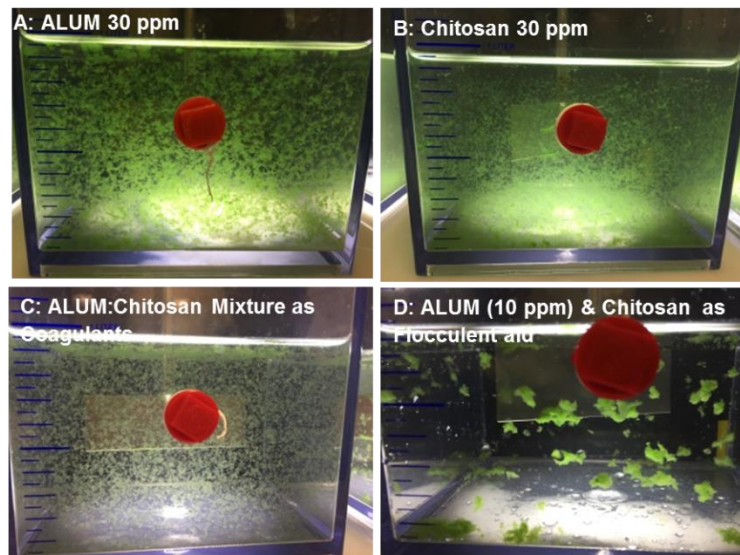
Figure 5: Pictures of well-grown flocs taken towards the end of flocculation process. Floc size for A(Alum 30 ppm)- 2.0 mm to 5.1 mm; B (chitosan 30 ppm)- < 3.0 mm; C (Alum:Chitosan mixture as coagulants)- 2.7 mm to 4.4 mm; D (Alum 10ppm & Chitosan as flocculant aid)- 3.8 mm to 12.7 mm

For the second set of the dual coagulation experiments (case 2), an alum dose of 10 ppm was added during the rapid mixing stage, and a chitosan dose was added during the slow mixing/flocculation stage (Figure 4D, Table 2). The microalgae removal efficiency was over 98%, as shown in Figure 4D. The optimum removal efficiency was observed for alum:chitosan (10 ppm:1 ppm), which then plateaued when overdosed with chitosan, with a sedimentation settling time of five minutes. Furthermore, larger flocs were observed, as shown in Figure 5 D. Clarified water was filtered using 0.45 micron filter, and the residual aluminium concentration was found to be 67 μg/L. The dual coagulation experiments resulted 10 times lower residual aluminium concentration than observed for alum coagulation.

The alum addition during the rapid mixing lowered the charge of suspended microalgae, and allowed microflocs to form, after which a slight amount of chitosan added during the slow mixing facilitated further charge neutralization and enhanced bridging between microflocs.

Similar experiments were performed using a fixed dose of 5 ppm ferric chloride coagulant, and a varied dosage of chitosan as a flocculant aid. 97% microalgae removal efficiency (Figure 4F) was achieved for the ferric chloride: chitosan (5 ppm: 2.5 ppm) dose levels, with a sedimentation time of five minutes. Clarified water was filtered using 0.45 micron filter, and the residual iron concentration was found to be < 1μg/L.

3.4 Mechanism model for Dual Coagulation for HABs in seawater:

The coagulation process took place after the effective elimination/lowering of the DLVO energy barrier, also referred to as *destabilization* [34]. An ionic concentration of sea water compressed the double layer around the microalgae particles. For low salinity waters, colloids can be destabilized by the addition of an indifferent electrolyte. Addition of indifferent electrolyte increases the ionic

strength of solution that has the effect of compressing the electrical double layer. Due to high salinity of the sea water, the counter ions are pushed closer to the surface the repulsion forces become easier to negate by van der Waals forces. Charge neutralization happened after the adsorption of a positively charged Al/Fe on the surface of microalgal particles. Charge neutralization produced an aggregation of microalgae particles to form bigger flocs. When chitosan was introduced during the coagulation stage, particle entrapment and bridging took place. These mechanisms contributed to a slight increase in microalgal removal efficiency (90%) when compared to the alum coagulation process, with a microalgae removal efficiency of 83% (Table 2). However, the flocs produced (Figure 5C) were similar to the flocs obtained for chitosan coagulation (Figure 5B), and required a longer (30 minutes) sedimentation time. During the dual coagulation, an overdose of chitosan led to charge reversal/re-stabilization, facilitated by reaction 2 of Figure 6. The process of charge reversal could be attributed to the effects of the rapid mixing, where a destabilized particle underwent a secondary adsorption of chitosan polymer by hindering the vacant sites of other nearby particles.
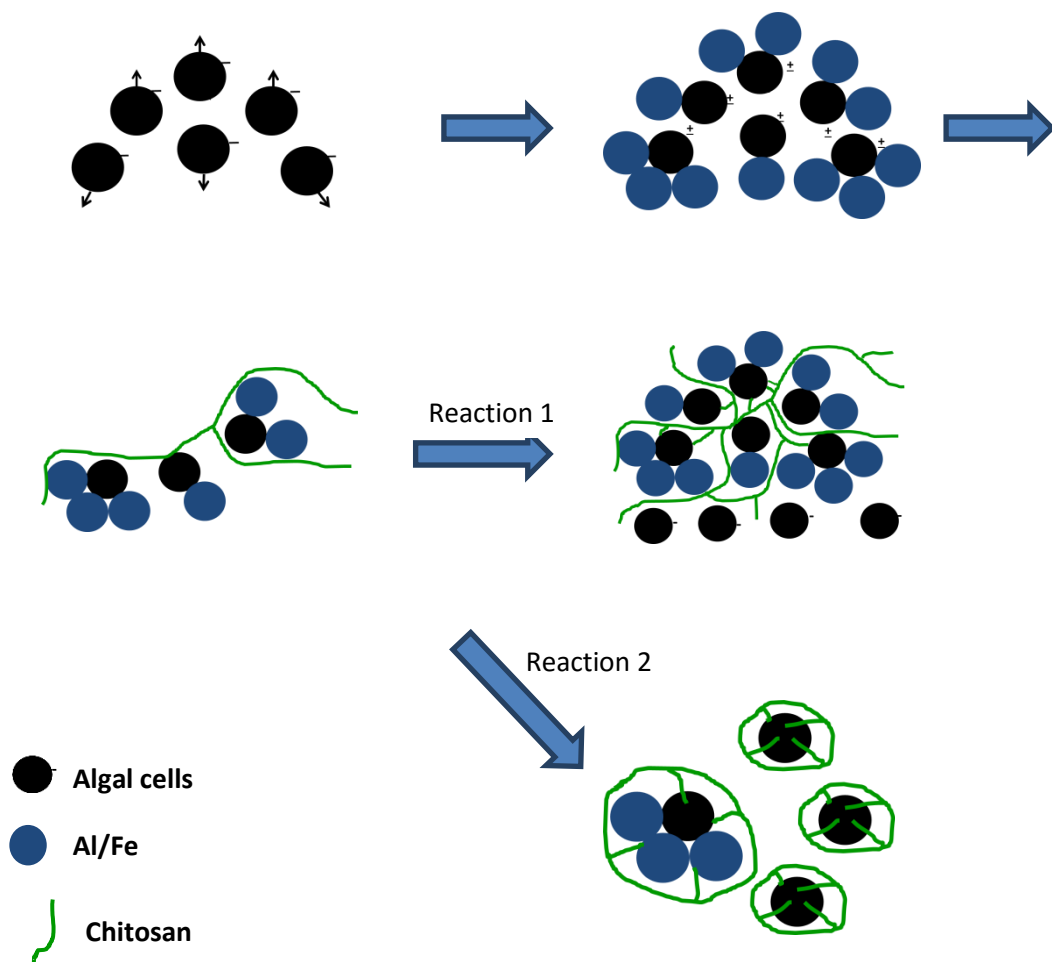
Figure 6: Mechanism model for dual coagulation. Reaction 1 Sweep Flocculation; Reaction 2 charge reversal/re-stabilization

In contrast, chitosan addition during flocculation stage (slow mixing) resulted in a macroflocs of size > 1cm (Figure 5 D), and the sedimentation time observed for this process was much shorter (five

13

minutes). This process was facilitated by a chitosan bridging of micro aggregates, and followed by a sweep flocculation, as shown in reaction 1 of Figure 6. The orthokinetic/macroscale flocculation induced the contact of particles through bulk fluid motion (gentle motion of fluid) and velocity gradients in the liquid. The dual coagulation approach resulted in a lower residual of aluminium/iron. Thus, the dual coagulation experiments were very efficient for microalgae laden seawater, as they resulted in a shorter process time, with low doses of alum/iron coagulants and chitosan as a flocculant aid. The C-F-S processes for seawater were governed by double layer compression, charge neutralization, bridging and colloidal entrapment mechanisms.

4 Conclusions

Individual coagulations using alum, chitosan and $FeCl_3$ resulted in reduced levels of microalgae in seawater feed for desalination. C-F-S experiments using $FeCl_3$ coagulant gave better process performance (20 ppm $FeCl_3$ dose, raw water pH, 30 min sedimentation time and 98% microalgae removal efficiency) when compared to alum and chitosan based individual coagulations. C-F-D process demonstrated improved process performance with a reduced coagulant dosage requirements and the process time (5 ppm $FeCl_3$ dose, raw water pH, 5 minutes flotation time). Despite of its improved performance, energy requirements of the C-F-D process are proven to be high, therefore alternate strategies were developed to improve the C-F-S process. The process time of the coagulation process was significantly reduced by the addition of chitosan as a flocculant aid. For dual coagulation using alum (10 ppm) as coagulant and chitosan (1ppm) as flocculant aid improved microalgae removal efficiency to 98% at a reduced process time of 5 minutes, thus making C-F-S process as attractive as C-F-D process. C-F-S processes for seawater were governed by double layer compression, charge neutralization, bridging and colloidal entrapment mechanisms. Moreover, the residual alum concentration in dual coagulation process using alum and chitosan was significantly reduced when compared to alum based individual coagulation. Further studies will be focused on economics of the above pre-treatment processes, and effects of microalgae on membrane performance during sea water desalination.

5 Acknowledgements:

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